Summary and Conclusion
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Isolation, purification and characterization of *Palamneus gravimanus* venom components were taken up with an intention to investigate into the biochemical nature of the venom components, their biological activity and therapeutic usefulness.

*Palamneus gravimanus* venom besides presenting chemical diversity not only continues to be an important source of lead compounds in medicinal chemistry programmes but also provide biochemical tools for mechanistic studies.

In the present investigation *Palamneus gravimanus* venom was subjected to cellulose acetate and agarose gel electrophoresis to investigate into the nature of the venom components and its subsequent purification. Agarose gel electrophoresis gave better separation in 0.2M Tris-HCl buffer (pH 8.5) compared to cellulose acetate electrophoresis. Electrophoretic data confirmed that the *Palamneus gravimanus* venom consists of both, basic and acidic proteins.

The same buffer system was employed for two step toxin purification. First step being fractionation of crude *Palamneus gravimanus* on a CM- Sephadex C-25 cation exchange chromatography immediately followed by sephadex G-50 gel filtration chromatography, which isolated a 4.5 ± 0.1Kd
pure toxin in an appreciable yield whose homogeneity and molecular weight was confirmed by SDS-PAGE and gel filtration chromatography using Sephadex G-75.

This toxin had an LD_{50} value of 2mg/kg body weight compared to the crude *Palamneus gravimanus* venom whose LD_{50} was about 800μg/kg body weight. This observation certainly strengthens the belief that synergistic action of other non toxic components enhances the toxicity of the pure toxin.

This purified toxin was subsequently tested on *Xenopus levis* oocytes, expressed human cloned potassium channel (hKv1.1) activity. It is reported for the first time in the present investigation that a nanomolar concentration of the purified toxin specifically blocks the hKv1.1 channel.

Isolation, purification and characterization of hyaluronidase enzyme with respect to its molecular weight and inhibition studies, is also reported for the first time in the present investigation. 25.6 fold purified hyaluronidase enzyme having specific activity of 6411.7 ± 117 TRU/min/mg with a yield of 39.2% was obtained in a two step process. Step one being fractionation of crude *Palamneus gravimanus* venom on Sephadex G-75 gel filtration chromatography followed by DEAE-Cellulose anion exchange chromatography. This purified enzyme showed a single band on Native PAGE confirming its purity and its monomeric nature. The isolated enzyme was 52 ± 1Kd with an appreciable yield, whose molecular weight was confirmed
by SDS-PAGE and gel filtration chromatography using Sephadex G-75. This enzyme showed thin white precipitin band in comparison to three multiple fused bands of the crude *Palamneus gravimanus* venom on Ouchterlony double immunodiffusion studies.

This isolated hyaluronidase enzyme had an optimum pH 4.5 and temperature 37°C respectively, and had a $K_m$ and $V_{max}$ value of 47.61 µg/ml and 1.49 µg/min. The enzyme was stable for one month at 0°C and its activity was enhanced by the addition of 0.15 M NaCl however, the enzyme activity was unaffected by the addition of thiol compounds like reduced glutathione, dithiothreitol, 2-mercaptoethanol, L-cysteine, Metal chelating agents like EDTA and urea did not show any significant inhibitory effect, while heparin at 60 I/U completely inhibited the enzyme activity. This was a very significant observation.

Probable important therapeutic application would be its combination with anticancer drugs and its inhibition by heparin in the promotion of wound healing in burns victims by restoring the victim’s hyaluronic acid levels.

Presently hyaluronidases are widely used in orthopaedics, surgery, ophthalmology, internal medicine, oncology, dermatology, and gynecology. Hence identification of a rich natural source like *Palamneus gravimanus* venom in the present investigation is noteworthy.
Demonstration of the antigenic nature of the crude as well as purified hyaluronidase enzyme is also a first report of its kind in the present investigation.

Demonstration of *Palamneus gravimanus* venom being a rich source of diagnostically important enzymes like 5' nucleotidase in the diagnosis of head and neck cancers and acetylcholinesterase in anaesthesia, while protease, phosphomonoesterase, L-amino acid oxidase being important in biological and biomedical research is advocated in the present investigation.

Development of a rat model study and correlation of certain biochemical parameter level changes in a dose dependant intramuscular administration of crude *Palamneus gravimanus* venom was reported for the first time in the present investigation. Toxic effects on specific organs like liver, kidney and heart were also studied for the first time in the present investigation. Selection of a rat model was intentional because of its homology to human beings. The observed changes in the biochemical parameters is intended to be an ideal guide to Clinicians, medical students, drug designers and forensic science experts. All the relevant data concerning the enzymic and nonenzymic parameters administered in control rats in different dosages before and four hours after injection is intended to establish a delicate correlationship between the cell, tissue and organ damage. Elevated serum levels of certain clinically important enzymic and
nomenclature was also observed for the first time in the present investigation.

Animal toxicity studies have immensely contributed to our understanding of not only, *Palamneus gravimanus* envenomation, but also has enabled us to develop animal models which could help in drug design and development, possibly to revert these damages to alleviate human sufferings in general and save precious human and animal lives in particular.